A Morphogenetic Study of Axial Patterns of Tracheid Differentiation in Douglas Fir

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INTRODUCTION

As a result of depletion of old-growth forests, young, fast-grown trees are being harvested for the manufacture of wood products. The quality of wood from these younger trees is very different from that of old growth. A higher proportion of the stem is juvenile wood in which anatomical structure changes markedly through successive growth rings. These changes in anatomical structure give rise to differential physical and mechanical properties within a piece of wood that seriously affect the processing of wood and its final properties.

Over the last few decades, forest management practices have changed, particularly in the direction of more intensive management. It should be possible to use forest management as a tool for the improvement of wood quality. However, currently our understanding of the processes of wood formation are insufficient to accurately predict effects of stand management.

Within juvenile wood of coniferous trees, the size of tracheids and thickness of tracheid walls increases with distance from the pith and with distance down the

stem. The cause of these patterns of variation is poorly understood. Although the patterns have been attributed to physiological gradients associated with the crown, there is also evidence that the cambium changes in response to morphogens through time. An understanding of what causes these patterns of variation is crucial for the prediction of the effects of stand management regimes on wood quality.

OBJECTIVES

Mature tracheid diameter and wall thickness depend upon rates and durations of the differentiation phases. In normal growth ring development, the seasonal changes in tracheid diameter and wall thickness are attributed primarily to changes in durations of differentiation. However, there is no information on the relative importance of rates and durations of differentiation in determining axial patterns of cell dimensions.

The present study was aimed at providing information on how rates and durations of wood production and tracheid differentiation vary along stems of *Pseudotsuga menziesii* (douglas fir). To achieve this, kinetics of cell production and differentiation were followed through the growing season at five stem levels in trees of three crown classes.

Specific objectives were 1) to examine possible differences in rates and durations of xylem cell production and differentiation along the stem, and 2) to examine the effect of crown class on axial patterns of variation in rates and durations of cambial activity along the stem.

LITERATURE REVIEW

Patterns of variation in the size of wood elements occur in softwood trees (Panshin and DeZeeuw, 1980). The two major patterns are (1) horizontal, from pith to bark, and across a growth increment; and (2) vertical, along the length of a stem in a given growth increment. Cells increase in size from pith to bark. Across a growth increment, radial diameter of cells decreases from early to latewood, and cell wall thickness increases. Along the stem, tracheid diameters and wall thicknesses increase down the stem to a maximum near the base of the live crown, and decrease to the base of the stem (Panshin and DeZeeuw, 1980). Consistent with these trends, percent latewood and wood density are commonly greater in the lower portion of a stem than higher in the tree.

CAMBIAL REACTIVATION

So far as is known, the cambium of temperate zone conifers is inactive in winter (Philipson, et. al., 1971). During the period of inactivity, the cambial

cells are radially compressed, with relatively thick walls. When growth is resumed, the cells swell and the walls become thin and more plastic (Wareing, 1958). Divisions first occur in the xylem mother cells adjacent to cambial initials, followed by divisions in the initials until a peak rate of cell production is reached a few weeks later (Bannan, 1954). At this stage the cambial zone is at its greatest width,the cells are large with thin walls and may be easily ruptured.

The position of first divisions within the stem is a subject of debate. Priestley (1930) found in several softwoods and hardwoods that cell division began beneath the buds in spring and spread basipetally through the tree. At about the same time, discovery and isolation of natural growth promoters, auxins, followed by the identification of the active substance, IAA, led to the formulation of the hypothesis that: 1) IAA was produced in active buds and elongating shoots, 2) IAA was transported basipetally and 3) IAA was the hormonal stimulus in cambial reactivation (Avery, et.al., 1937). Wareing (1951) supported this theory from bark ringing experiments in which cambium below a ring remained inactive. This suggested blockage of a polarly transported substance involved in stimulating cambial reactivation.

Other support for this theory was found in the correlation between bud break and onset of cambial activity below the buds (Ladefoged, 1952). In Picea sitchensis, Denne (1979) found basipetal spread of cambial reactivation in branches. In the main stem, earlier activation occurred at a midcrown position, but this was interpreted as being due to the earlier arrival of a stimulus in the main stem from branches of the lower, rather than upper, crown. She found a close correlation between the pattern of cambial reactivation in the stem and the pattern of bud break in the crown.

The basipetal spread of cambial reactivation has been challenged by several authors. Savidge and Wareing (1981) observed cell divisions before bud break in the main stem of the lower crown and bases of lower branches of Pinus contorta, before divisions were apparent in the upper stem. They concluded that auxin supplied by mature needles was sufficient for cambial reactivation and new xylem formation, although continued cambial activity depended on extending shoots and developing leaves.

Whitmore and Zahner (1966) found that cambium of Pinus resinosa became active even if trees were disbudded before terminal growth began. Wort (1962), cultivating dormant cambium of several species in vitro, found resumption of activity in the absence of added

growth promoters when tissues were exposed to temperatures around 25 C. He suggested that auxin reserves in tissues adjacent to the cambium may be liberated by temperature increase. This observation could account for the reports of initiation of cambial division in the absence of buds. Dormant trees have been shown to initiate cambial activity under heat bands in the absence of shoot growth (Savidge and Wareing, 1981).

Conflicting reports by these investigators may be due to measurements of cambial activity, or the definition used. Denne measured the first formed tracheid. Savidge used the first division to mark cambial reactivation, and others have used the fact that bark slipped to indicate reactivation.

Denne (1979) showed the rate of progress of cambial reactivation in Picea sitchensis to be directly related to tree crown class. She found rate of reactivation to be slower in suppressed than co-dominant trees. She was also able to show that tracheid production began earliest in the lower crown, but progressed at a faster rate higher in the crown.

CESSATION OF CAMBIAL ACTIVITY

The pattern of radial growth in the stem may vary with the degree to which the tree is suppressed. Larson (1973) reported that in open stands, radial growth continued later into the season at progressively lower positions in the stem. However, in Picea sitchensis, Denne (1979) reported cessation of cambial activity did not vary in trunks of trees which were not suppressed, although in several suppressed trees there was a shorter duration of cambial activity below the crown.

Cessation of cambial activity has been attributed to indirect effects of water stress through the tree's crown (Larson, 1964, 1969; Denne and Dodd, 1981) and to decreasing endogenous auxin levels (Larson, 1962, 1964; Savidge and Wareing, 1984). In conifers, cessation of cambial activity due to decreasing auxin levels seems doubtful since mature leaves have been reported to produce auxin, and high levels of auxin have been reported in the dormant cambium (Little and Wareing, 1981; Savidge and Wareing, 1981).

Increasing levels of growth inhibitors might be expected to play a role in cambial dormancy. However, seasonal levels of abscisic acid (ABA) showed no significant increase as shoots, needles, and cambium

ceased growing (Savidge and Wareing, 1984). Cambial IAA levels reached a maximum in midsummer and remained high until autumn inactivation of cambium when they decreased. Little and Wareing (1981) had earlier cast doubt on a role for ABA in controlling cambial dormancy. After elaborate experimentation involving measurements of IAA and ABA levels under various conditions (disbudding, girdling, defoliating, droughting, changing photoperiod, and exogenous IAA applications), they concluded that the change from cambial activity to rest at the end of a season is due to the development of an inability to respond to IAA, not a deficiency in IAA supply. In spring, this ability to respond is regained.

PATTERNED VARIATION: POSSIBLE CAUSES

I. Cambial Age

Differential cambial response was the subject of an earlier study by Zajaczkowski (1973), where interactions of IAA and other growth regulators in combination with varying concentrations of sucrose were studied in isolated stem segments collected through the season. None of the variables studied could account for the observed seasonal variation in number of cells produced by the cambium in response to IAA. Interestingly,

segments taken from different heights in the tree stem showed different responses of cambia to IAA, in terms of cell numbers produced, suggesting an age-related phenomenon.

Beckwith and Shackelford (1976) had difficulty correlating variations in cell size as measured by a crown growth index. They used cambial age as a factor to modify their crown growth index, and obtained good correlations between this modified growth index and variations found in cell diameter and wall thickness through the stem. They concluded that an age-related factor was involved in controlling wood cell dimensions but, contrary to Zajaczkowski's (1973) suggestion that cell number is related to cambial age, they supposed crown growth predominated in control of the number of cells produced each season. Farrar (1961) found that ring width increases from the apex to the heaviest part of the crown. Below the crown, ring width may increase or decrease; in stand grown trees, it decreases and the effect is more pronounced as crowns become smaller. In vigorous trees with large crowns, ring width may increase below the crown. In severely suppressed trees, there may be no annual increment in the lower bole (Denne, 1979).

Poor correlations between some aspects of cell morphology and crown size (Dodd, 1985) or position

(Denne, 1979) furthur support the concept that axial gradients of morphogens alone are unlikely to explain axial patterns in cell morphology. It seems likely that concentration gradients of growth regulators may be interacting with intrinsic properties of cambium to produce the anatomical patterns which occur in a tree. Clearly, the crown plays a role in regulation of wood production.

Other evidence for a differential cambial response due to cambial age came from Savidge's (1983) work. He applied IAA to different-aged, disbudded, and defoliated cuttings of Pinus contorta, and observed cambial cell divisions in one- year-old cuttings, finding decreasing activity farthest from the point of application. Two-year-old cuttings exhibited cell divisions only at the uppermost position, with less intensity than that of the same position in one-year-old cuttings; 4- to 6-year-old cuttings showed no cambial cell divisions.

II. Hormonal Theory

Manipulation of such factors as photoperiod and drought has led various researchers to conclude that radial cell diameter and wall thickness are independently controlled (Larson, 1960, 1962; Wodzicki, 1971; Skene, 1969). Larson (1969) manipulated auxin and

sucrose concentrations to produce tracheids with various combinations of radial diameter and cell wall thicknesses. These results led him to suggest that the interaction between auxin and sucrose during cell differentiation determines the tracheid type to be produced. This contributed evidence for the concept of hormonal control over growth ring formation (Wareing, 1958; Larson, 1960, 1962).

The concept of hormonal control relates production of auxin associated with shoot extension and leaf development in the crown, with the formation of tracheids having large radial diameters of the earlywood type. According to Larson (1969), the cessation of shoot growth is accompanied by reduced levels of auxin and reduced tracheid diameters. Larson also suggested that the phase of cell wall thickening is highly correlated with current net photosynthate production and distribution. He proposed that assimilates are used in shoot elongation in the early season and are made available for use in wall thickening when shoot elongation ceases, resulting in increased wall thickening later in the season.

Even though Larson's hypothesis has gained supportive evidence, there are conflicting reports. Larson (1962) reported high auxin levels to be associated with shoot growth and earlywood formation in

Pinus resinosa. Studies by Balatinecz and Kennedy (1968) on Larix decidua supported Larson. However, Wodzicki (1971) found extractable auxin levels to be relatively constant through the season in Pinus sylvestris, thus reduced tracheid diameters through the season could not simply be attributable to lack of auxin availability as Larson's hypothesis proposed.

Balatinecz and Kennedy (1968) reported incresed tracheid diameters after lateral applications of indolic compounds in Larix decidua. Although Denne and Wilson (1977) found increased cell diameters and wall thickness when exogenous IAA was applied to intact shoots, they found no effect of exogenous IAA on tracheid dimensions, in disbudded shoots. They suggested that perhaps exogenous IAA acted synergistically with endogenous growth substances.

Hejnowicz and Tomaszewski (1968) showed IAA to be the principal limiting factor inducing xylem formation but also pointed out that exogenously applied IAA alone only partially substituted for the growing apex. A joint application of IAA, GA, and cytokinins induced normal wood formation. Since comparable amounts of these substances were found in growing buds and cambium of Pinus sylvestris, they accepted that these substances work together in the control of wood formation.

Studies of application of exogenous growth

regulators suggest the possibility that factors other than photosynthetic products affect wall thickening. An increased wall thickness was reported after application of IAA in Pinus elliottii by Nix and Brown (1987) and in two species of Picea by Denne and Wilson (1977). In liquid stem explant culture, Sheriff (1983) found cell wall thickness to increase with concentrations of exogenously applied IAA up to 20 mg/l, resulting in compression wood-type tracheids at the higher concentrations.

Studies of rates and durations of cell wall thickening have shown that increase in wall thickness through the season is not simply due to availability of carbohydrates. Increase in secondary wall thickening has been associated with variation in the duration of wall thickening (Skene, 1972; Denne, 1974; Nix and Brown, 1987). However, Denne (1976) found conditions that favored assimilate supply affected the rate of wall thickening rather than its duration.

In an experiment involving apical control of xylem formation and the response of differentiating tracheids, Porandowski (1982) suggested that regarding growth and differentiation, auxin may indirectly induce structures involved in cell wall formation. He proposed that the presence of auxin during radial growth and maturation makes possible the expression of cellular potential in

terms of the rate of wall formation. Reduction of auxin supply resulted in a delay of autolysis of protoplasts which marks the end of the wall deposition phase of differentiation. This implies that auxin specifies positional information across the growth ring to differentiating cells for autolytic processes. This type of information transfer has been proposed by Zajaczkowski and Wodzicki (1978), who suggested that the position at which a cell looses its protoplast is determined by a vectorial field resulting from waves of polarly transported auxin in the cambial zone. This mechanism could be significant in the regulation of the transition from early- to late-wood.

Wodzicki and Wodzicki (1980) discovered that apically applied abscisic acid reduced the amplitude of the morphogenetic auxin wave. They postulated that seasonal accumulation of abscisic acid could delay autolysis by its inhibitory effect on phenomena associated with polar auxin transport. Based on this and their earlier work, they proposed a mechanism involving natural inhibitors of auxin, such as ABA, for control of initiation of latewood in conifers.

There are contradictory hypotheses concerning the control of tracheid size along an axis. Larson (1969) proposed that gradients of tracheid diameter through the stem are positively regulated by parallel gradients of

auxin.

Alternatively, Aloni and Zimmermann (1983) proposed an inverse relationship between auxin levels and vascular element dimensions as part of their 'six point hypothesis'. In studies with the angiosperm Phaseolus vulgaris they found the rate of cell differentiation determined the final diameter of vessels. Rate was positively correlated with auxin concentration such that a rapid rate of differentiation caused vessels to be numerous and small near the point of IAA application. Lower auxin concentrations basipetally resulted in a slow rate of differentiation which produced fewer and larger diameter vessels. These findings supported their hypothesis that auxin controls size and density of vessels along a plant axis through basipetal polar flow. They acknowledged that a signal which travels along the axis in the form of waves may subject the cells to intermittent gradients. Such waves could convey detailed information to cells concerning their position within the plant, as well as across the growth ring. More recently, Saks and Aloni (1985) studied tracheid differentiation in the hypocotyl of Pinus pinea, finding evidence in support of the six-point hypothesis.

KINETICS OF TRACHEID DIFFERENTIATION

The physiological basis for seasonal changes in tracheid diameter and wall thhickness is of great interest because these changes influence wood quality. Radial diameters are greatest early in the growing season and decrease gradually. The last formed tracheids are radially flattened and similar in shape to the cells of the resting cambium. Cell wall thickness is minimal in early formed tracheids and gradually increases until a maximum is reached near the season's end, and often decreases again in the last formed cells (Wodzicki, 1962).

Investigations of tracheid production and differentiation in conifers suggest that changes in the rate of cell division, associated with environmental fluctuations, were followed by changes in the numbers of cells in each of the two differentiating zones and, thus, by changes in the durations of time spent by cells in each zone (Ford, et.al., 1978). In Pinus radiata, Skene (1972) found that the increase in cell wall thickness through the season was associated with a decrease in cell diameter such that the wall material per tracheid varied little through the season. The decrease in radial cell diameter through the season was

associated with a decrease in the duration of radial expansion, and the increase in wall thickness was associated with increased duration of wall thickening. Skene concluded, since the volume of cell wall material was relatively constant through the season, that the increased duration of secondary wall thickening was associated with a decrease in the rate of secondary wall deposition.

Denne (1972) also found increased cell diameters to be due to an increase in the duration of the phase of radial cell expansion rather than to a change in rate. However, contrary to this conclusion, earlier work by Wodzicki (1971) suggested that variation in radial cell diameters was probably dependent on seasonal changes in the rate of growth.

Rate of formation of cells has been found to decrease basipetally as radial cell diameters increase (Aloni and Zimmermann, 1983; Denne, 1979). Aloni and Zimmerman concluded that auxin was involved in control of the size of xylem elements through the stem. However, Denne (1972) proposed that rates of xylem formation did not reflect growth regulator gradients, for though the rate varied within the plant, cell number increased at a constant rate at each stem level suggesting rate of cell production is a function of age of the apical meristem at the time of origin of the

cambium rather than current distance of the cambium from the apex.

Nix and Brown (1987) reported that autolysis of protoplasts can be artificially induced by increased exogenous applications of IAA and that this application could also decrease the duration of radial cell expansion.

MATERIALS AND METHODS

SAMPLING

Douglas fir trees were collected successively through the 1986 growing season from the Boggs Mountain State Demonstration Forest, Lake County, California, elevation 762 m. The forest is of an interior coastal mixed conifer type with ponderosa pine predominating. This site is characterized by hot and dry summers and winter rains. Monthly temperature and rainfall conditions for the 1986 season are shown in Table 1.

Trees approximately 6 m in height were selected from three crown classes (Figure 1). Selection of crown classes (CC) was on the basis of the length of branch-free bole. Within each crown class, trees were selected for uniform basal stem diameter.

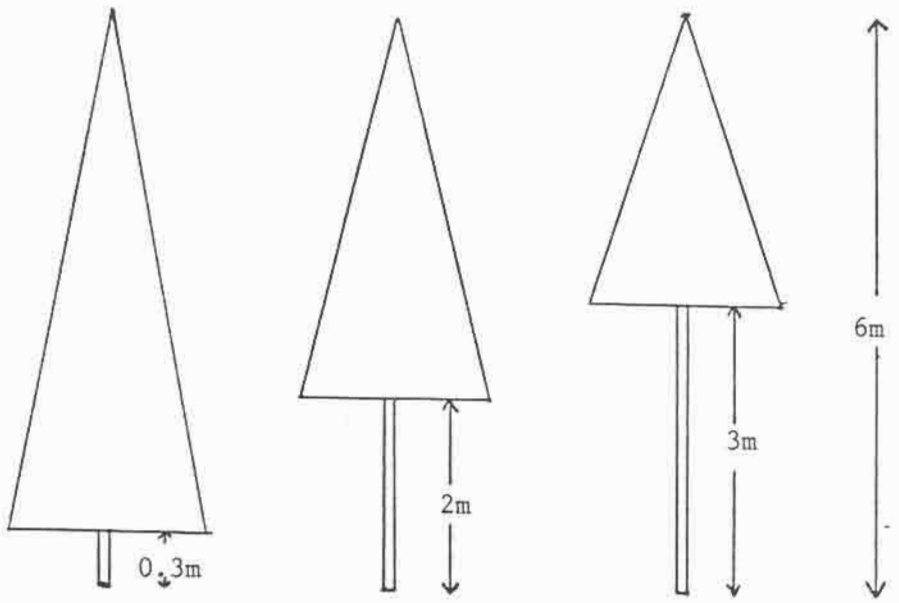
Five trees in each crown class were felled at approximately 3 weekly intervals from Februrary to September, 1986 (Table 2). Segments were cut at 1 meter intervals along the stems with the first sample cut from the top of the 1985 increment of growth. The stem segments were stored overnight at 4 C. Samples were cut from the stem segments on a band saw, avoiding branches

TABLE 1. MONTHLY HIGH AND LOW TEMPERATURES AND CUMULATIVE MONTHLY RAINFALL AT BOGGS MTN STATE DEMONSTATION FOREST DURING THE SAMPLING PERIOD, 1986

	TEMPERATURE				RAINFALL
	LOW		HIGH		
	(C)	DATE	(C)	DATE	(cm)
FEB	-2.8	11 FEB	23.9	27 FEB	67.5+
MAR	-1.1	14 MAR	25.6	27 MAR	30.6
APR	0.6	14 APR	27.2	22 APR	2.7
MAY	0.0	6 MAY	31.1	31 MAY	3.3
JUN	6.1	18 JUN	33.3	25 JUN	0.0
JUL	12.8	4 JUL	33.3	21 JUL	0.0
AUG	NO DATA AVAILABLE				0.0
SEP	4.4	23 SEP	35	8 SEP	3.8

Figure 1

BASIS OF CROWN CLASS SELECTION



CC1 CC2 CC3

Trees were selected on the basis of length of the branch-free bole ±0.2m.

CC1 = Crown Class 1

CC2 = Crown Class 2

CC3 = Crown Class 3

TABLE 2. DATES OF TREE HARVEST

DAY of STUDY	HARVEST DATE
0	28 FEB 86
14	14 MAR 86
28	28 MAR 86
46	15 APR 86
75	14 MAY 86
95	3 JUN 86
118	26 JUN 86
139	17 JUL 86
160	7 AUG 86

and severe compression wood, and fixed in Formaldehyde-Acetic acid-Alcohol (FAA) for a minimum of one week. These were then trimmed with a razor blade to approximately 0.5 cm x 0.2 cm in cross-section, and 0.5 cm in length.

SAMPLE PREPARATION

The trimmed samples were rinsed briefly in deionized water and immersed in a 10% solution of ethylenediamine for three days to soften the wood according to the method of Carlquist (1982). They were then dehydrated according to Johansen (1940) and embedded in Paraplast, a wax/plastic embedding medium (Monoject, Inc., St. Louis, Mo).

Transverse sections were cut at 16-20 u on a rotary microtome and were floated on microscope slides which were prepared by coating with a thin film of Haupt's adhesive, and two drops of 3% formaldehyde in water. The slides were warmed for about five minutes to initiate the drying process, then left in a cool dust-free place to air dry overnight before staining. The stain and counterstain used were safranin O and fast green FCF (Johansen,1940). Sections were permanently

mounted with Permount (Braun-Knecht-Hermann Co., San Francisco, Ca.) and weighted until dry.

MICROSCOPIC CRITERIA

Total numbers of cells were counted in ten radial files across the current season's growth. These total counts were divided into four phases of development as adapted from Skene (1969):

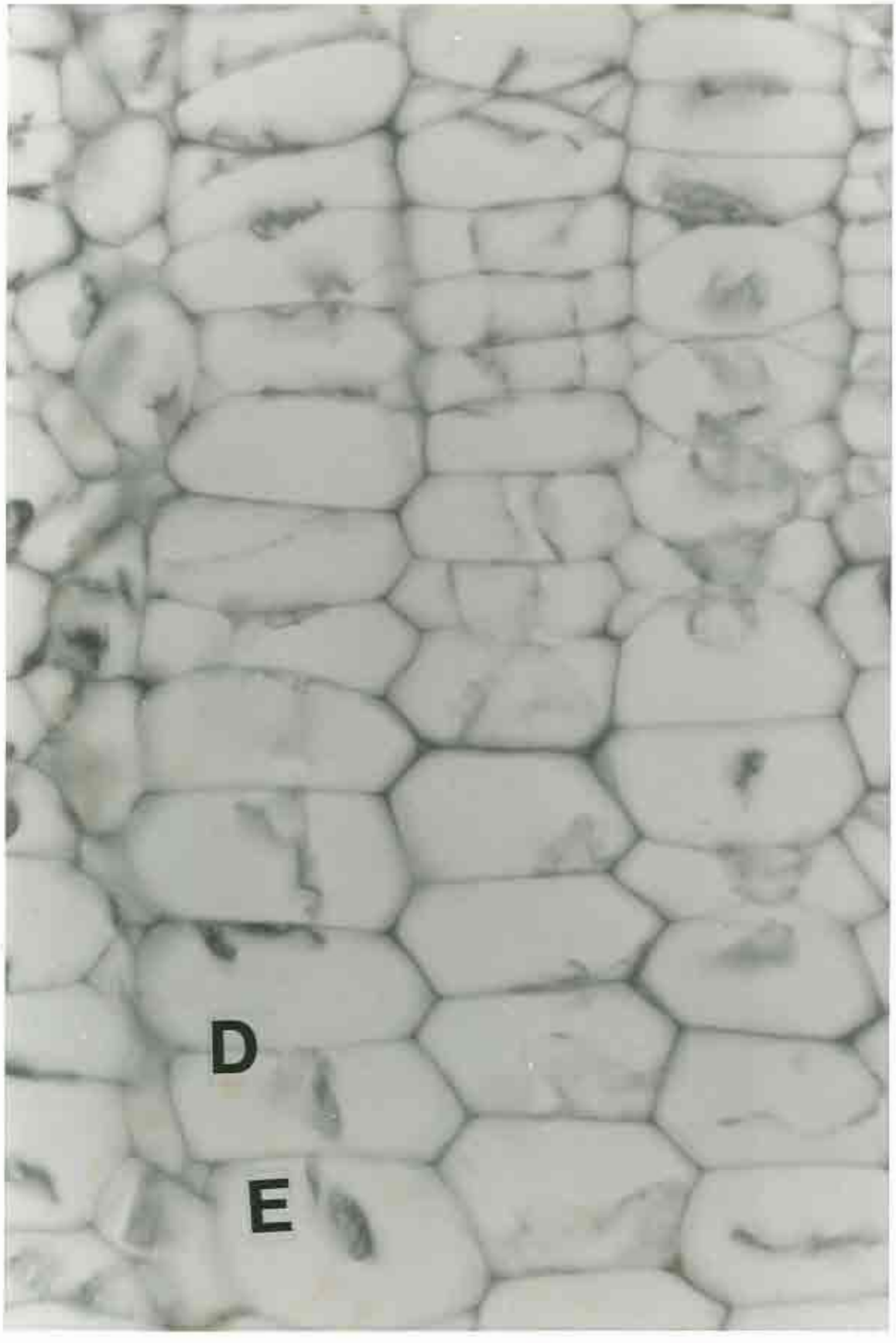
Dividing phase (D) - distinguished by the presence of thin tangential cell walls and compact radial dimensions, and by the absence of birefringence under polarized light. This zone included xylem mother cells which were commonly identified in pairs with thin intervening tangential walls (Figure 2).

Radial expansion phase (E) - distinguished by the absence of birefringence under polarized light, and absence of paired xylem mother cells commonly seen in the dividing zone. Also distinguished by the increased radial diameter of cells and irregular cell outlines.

Secondary wall depositing phase (W) - distinguished by the presence of cytoplasm in the cell lumen, and

FIGURE 2: DELIMITATION OF DIVIDING CELLS FROM EXPANDING CELLS

Radially flattened cambial cells and xylem mother cells, commonly occurring in pairs with thin tangential walls, are included in the cambial zone (D). Obviously radially enlarged cells with more irregular walls are included in the expanding zone (E).


D
E

presence of the secondary cell wall, yet absence of the S3 layer, as evidenced by polarized microscopy. In cases of severe compression wood where the S3 layer may not be visible even under polarized light, or because of poor specimen preparation, the differential staining of lignin by Safranin and cellulose by Fast green was also used in differentiating this phase.

Mature phase (M) - distinguished by the presence of the S3 layer of the secondary wall and the definite absence of cytoplasm from the cell lumen.

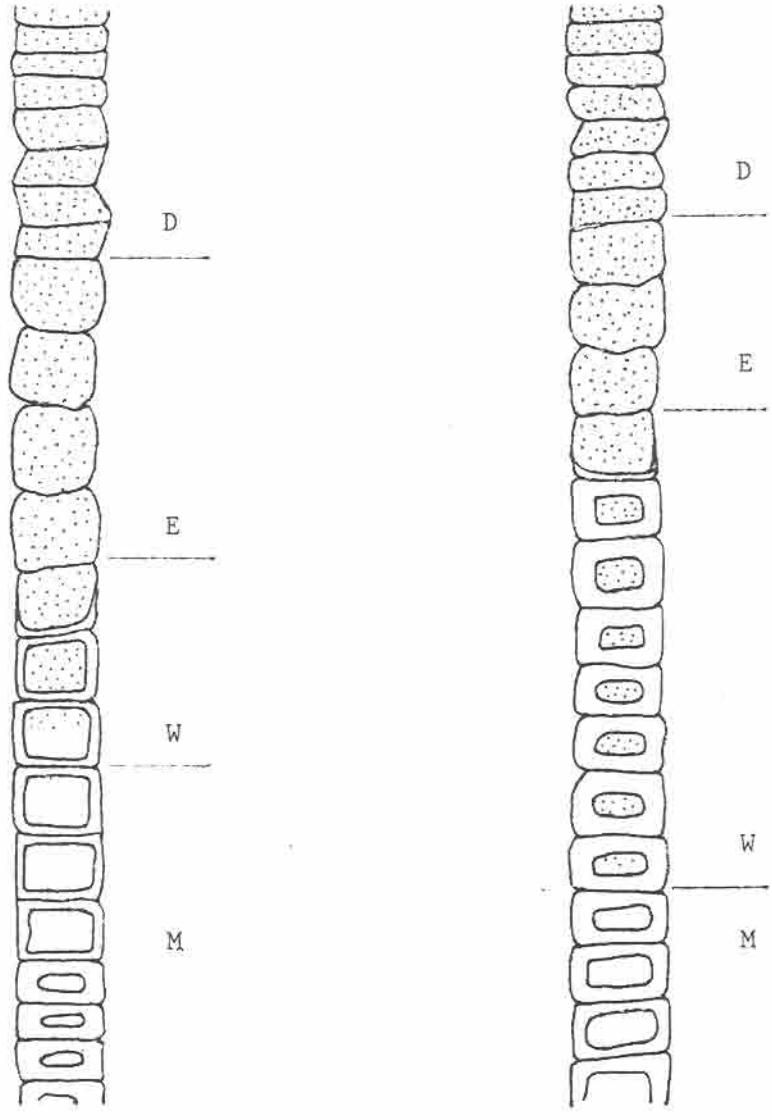
Figure 3, adapted from Wodzicki (1971), is a diagramatic representation of the above described zones.

In each file, the radial diameter of a radially flattened cell in the cambial zone, the last radially enlarging tracheid and the tangential wall thickness of the first fully mature tracheid were measured in order to determine the rates of cell expansion and cell wall deposition.

DATA ANALYSIS

For each tree, mean numbers of cells in the four phases were calculated from ten radial files. Best fit polynomial functions (up to the third degree) of cell number in each of the four phases were computed against

FIGURE 3: ZONES OF DIFFERENTIATING XYLEM



Stippling indicates cellular contents.

D = cambial cells and dividing mother cells

E = radially expanding cells

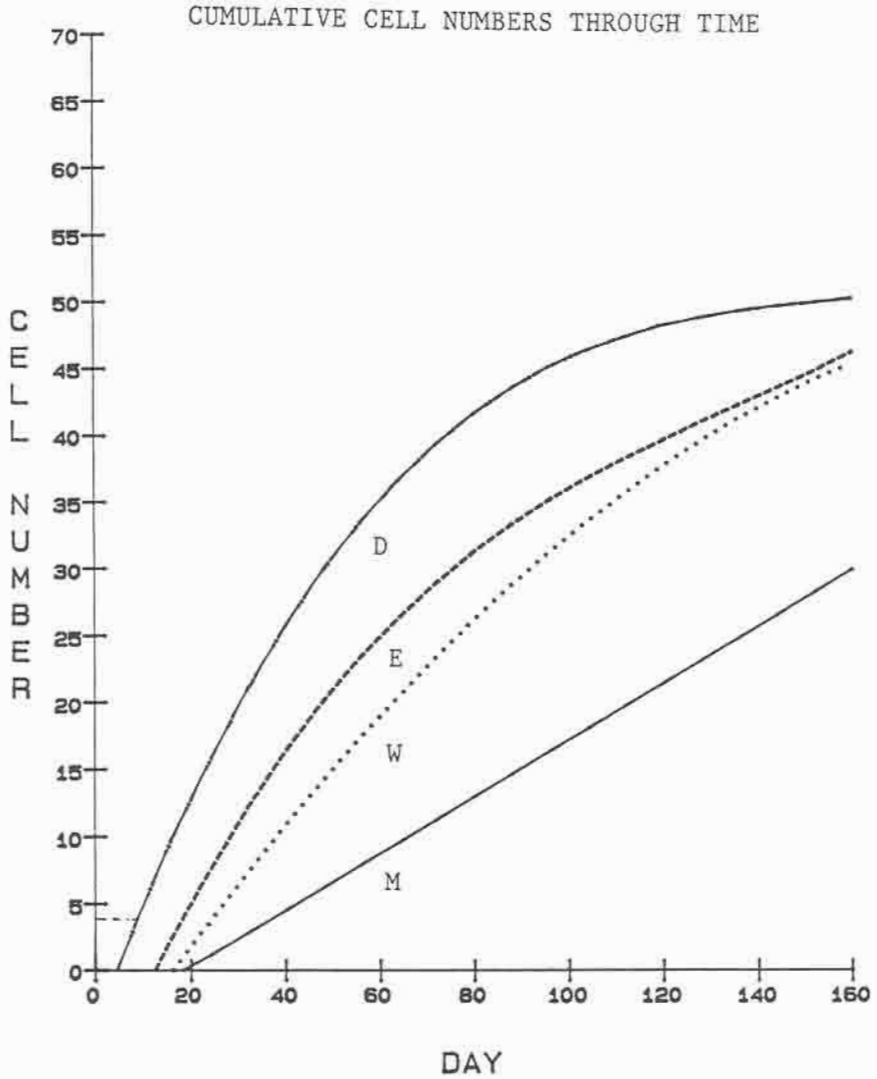
W = cells depositing secondary wall material

M = mature tracheids (no cellular contents)

time for the five stem positions in trees of the three crown classes. In all cases, a linear function was the best fit for mature cell numbers, and a second degree polynomial for the numbers of cells depositing secondary walls. Second and third degree polynomials were used for the dividing and expanding stages. To obtain cumulative cell numbers through time, functions for the successive stages from mature tracheids through wall thickening, radially expanding and cambial cells were added (Figure 4). The distance between two curves for a particular cell represents the duration (in days) of that cell in the phase bounded by the two curves (Whitmore and Zahner, 1966). The equations representing boundaries of differentiating zones (legend, Figure 4) were solved for every fifth cell using linear, quadratic, and cubic equations expressed as functions of Y. For each fifth cell, values for bounding curves were subtracted to obtain the duration a cell spent in a particular phase. The duration of time spent in each zone could then be plotted for each fifth successive tracheid formed through the season (Figure 5).

The rate at which cells expanded was calculated by subtracting the cambial cell diameter from the fully expanded cell diameter and dividing by the duration of time (in days) spent in the expanding zone (Denne, 1972). The rate of secondary wall thickening was

FIGURE 4



——— f (x) = .21166x −3.943
..... f (x) = −.00119x*2 +.5256x −8.294
---------- f (x) =.0000123x*3 −.005x*2 +.834x −9.82
——— f (x) = .0000123x*3 −.00586x*2 +.963x −4

Functions were fit to cell numbers in each differentiating zone. Functions for successive stages of development were added to obtain cumulative cell numbers.

D = cambial cells and dividing mother cells
E = radially expanding cells
W = cells depositing secondary walls
M = mature tracheids

calculated by dividing final wall thickness of the cell by the duration spent in the secondary wall thickening phase.

STATISTICAL ANALYSIS

For rates and durations of both cell differentiation phases, a two way ANOVA was employed to determined effects between stem positions and crown classes and whether interaction occurred between the two factors. Where significant differences or interaction occurred, Tukey's (Q) test (Miller, 1986), at a probability level of 0.05, was used for multiple comparisons to determine the location of the differences. Replications of rate or duration used were the values obtained through the season.

RESULTS

REACTIVATION OF THE CAMBIUM

The cambium was active in the uppermost sampling position for all trees at the first harvest date, 28 Feb 1986. Cambial activity was determined by the presence of one or more cells having radial diameters greater than radially flattened cells to their phloem side. In crown class 1 (CC1) activity was confined to the uppermost sampling position (Table 3). In crown class 2 (CC2) there was evidence of cambial activity in the four uppermost positions, and in crown class 3 (CC3), in the two uppermost positions.

By 14 Mar, 1986, day 14 of the study, there were radially expanding cells throughout all trees (Table 3). There was no obvious trend down the stems in numbers of cells undergoing expansion, with the possible exception of CC3 where cell number decreased basally. Among crown classes, expanding cell numbers were greatest in CC1 trees and successively fewer in CC2 and CC3 trees.

Also, by day 14, in the two uppermost stem positions of CC1 and in the uppermost position of CC2, some cells had begun depositiong their walls (Table 3).

TABLE 3.NUMBER OF CELLS IN THE CAMBIAL AND DIFFERENTIATING ZONES AT BEGINNING AND END OF THE SEASON IN DOUGLAS FIR TREES

		CAMBIAL ZONE			EXPANDING ZONE		
	DAY:	0	14	160	0	14	160
CC1	POSITION 1	5.3	9.8	5.3	0.4	4.3	1.5
	POSITION 2	4.0	12	6.6	0.0	6.0	1.9
	POSITION 3	5.7	15	6.4	0.0	8.0	2.3
	POSITION 4	5.3	13	5.8	0.0	6.0	1.2
	POSITION 5	6.3	11	5.5	0.0	5.2	0.4
CC2	POSITION 1	6.0	8.0	5.0	1.0	3.0	0.8
	POSITION 2	6.0	9.0	5.0	1.3	4.0	1.0
	POSITION 3	6.0	10	5.8	1.2	3.0	1.0
	POSITION 4	4.0	10	5.8	1.3	3.5	0.3
	POSITION 5	6.0	8.0	5.9	0.0	3.0	0.7
CC3	POSITION 1	8.0	8.0	4.4	1.6	1.6	0.9
	POSITION 2	7.0	9.0	5.5	1.0	2.0	0.8
	POSITION 3	6.2	6.0	5.0	0.0	1.0	1.0
	POSITION 4	5.0	5.0	5.0	0.0	1.0	0.9
	POSITION 5	5.7	6.0	5.0	0.0	0.5	0.5

WALL DEPOSITION ZONE

		DAY: 14	28	46
CC1	POSITION 1	1.2	4.1	7.2
	POSITION 2	0.5	3.5	8.7
	POSITION 3	0.0	3.7	7.1
	POSITION 4	0.0	4.2	10.7
	POSITION 5	0.0	3.7	9.3
CC2	POSITION 1	0.8	7.0	10.0
	POSITION 2	0.0	5.0	12.0
	POSITION 3	0.0	3.0	7.0
	POSITION 4	0.0	3.0	11.0
	POSITION 5	0.0	8.3	10.0
CC3	POSITION 1	0.0	1.0	3.5
	POSITION 2	0.0	0.0	7.0
	POSITION 3	0.0	0.0	7.0
	POSITION 4	0.0	0.0	10.0
	POSITION 5	0.0	0.0	11.0

It was not until day 28 that cells in the uppermost position of CC3 had begun wall deposition.

PRODUCTIVITY AND CESSATION OF CAMBIAL ACTIVITY

By the end of the season, total cell numbers in radial files were greatest in CC1, and successively fewer in CC2 and CC3 (Table 4). Along the stem, numbers of cells were greatest at the uppermost sampling position for all crown classes and decreased with successive stem positions basally.

On the last harvest date, 7 August, 1986, the width of the cambial zone in all trees was 4 to 7 cells (Table 3). Cambial divisions, thus, had probably ceased. Some cells were still undergoing radial expansion on this date. In CC1 trees, 1 to 2 cells were undergoing radial expansion at positions 1-4. In all other trees and positions, mean numbers of cells undergoing expansion were less than 1. This was essentially the same level of activity that had been observed on the previous harvest, 17 July, 1986.

TABLE 4. TOTAL NUMBER OF CELLS PRODUCED IN RADIAL FILES OF DOUGLAS FIR TREES

	CC1	CC2	CC3
POSITION 1	105	85	60
POSITION 2	105	75	55
POSITION 3	95	70	45
POSITION 4	80	55	45
POSITION 5	65	45	30

SEASONAL ANALYSIS

DURATION IN THE CAMBIAL ZONE

Throughout the stems of all 3 crown classes, the fifth cells produced spent approximately 10 days in the cambial zone (Figure 5). The time a cell spent in the cambial zone increased through the season.

Seasonal variation in duration within the cambial zone in CC1 was minimum for upper stem positions (ranging from 10 to 15 days), and increased down the stem (ranging from 10 to 35 days at the base of the stem). Trends in duration among stem positions were similar in CC2. In CC3, the seasonal increase in duration was greater for all stem positions than in the other two crown classes. A significant effect of stem position on duration in the cambial zone was found ($p<0.024$). However, there was no significant effect ($p<0.075$) of crown class (Table 5).

RADIAL CELL DIAMETERS

Radial diameters of tracheids that had just completed expansion were measured at each successive

TABLE 5: SIGNIFICANCE LEVELS OF CELLULAR DIFFERENTIATION PROCESSES IN DOUGLAS FIR AS DETERMINED BY TWO WAY ANALYSIS OF VARIANCE

	Crown class	Position
Duration in cambial zone	0.024	0.075
Cell diameter	0.317	0.055
Duration of radial expansion	0.013	0.001
Rate of radial expansion	0.0001	0.334
Cell wall thickness	0.88	0.087
Duration of wall deposition	0.0001	0.0001
Rate of wall deposition	0.0001	0.0001

harvest. In all crown classes, diameters were maximum early in the season and decreased across the growth ring to a minimum at the last sampling date (Figure 6). Although cell size tended to be maximum early in the season, the first formed cells were commonly smaller than those formed a little later. Radial diameters of the last cells formed were similar to those of resting cambial cells.

Differences in radial cell diameters among stem positions were smaller in CC1 than in the other crown classes. Diameters were smallest in the uppermost stem position in the early part of the season. Tracheid diameters were greatest in position 2. In CC2 and CC3 tracheid diameters tended to increase down the stem, however, no significant positional effect was detected ($p<0.055$). Among crown classes, tracheid diameters were smallest in CC3, however, the difference in diameters between crown classes were not significant ($p<0.317$).

DURATION OF RADIAL EXPANSION

The duratiion of cell expansion increased throughout the season to a maximum and then decreased (Figure 7). The peak in duration tended to occur later in CC3 (about late May) than other crown classes (early to mid May).

The duration of cell expansion tended to increase down the stem (Figure 7). Since durations increased more rapidly up to the peak duration in lower stem positions, greatest differences among stem positions occurred at this time. Analysis of variance showed a highly significant ($p<0.001$) effect of stem position on duration of expansion (Table 5).

Among crown classes, duration of expansion was greater in CC3 than the other two crown classes, mainly due to the very long durations at the base of the stem (Figure 7; Table 5).

RATE OF RADIAL EXPANSION

Among stem positions there was no clear trend in the rate of radial cell expansion, although in CC2 and CC3 the lower stem positions tended to have lower rates (Figure 8). Across the ring, the rate of expansion was not found to differ between stem positions ($p<0.334$).

Analysis of variance showed a highly significant ($p<0.0001$) effect of crown class on rate of cell expansion (Table 5). Mean rates were greatest in CC1 and least in CC3 (Table 6). Differences were mainly due to higher rates of cell expansion later in the season in CC1.

TABLE 6: MEAN DATA FOR CELL DIMENSIONS AND NUMBERS OF CELLS IN VARIOUS DIFFERENTIATING ZONES

POSITION		DIAMETER (u)	DURATION OF EXPANSION (days)	RATE OF EXPANSION (u/day)
1	CC1	15.03	5.08	2.22
1	CC2	15.50	5.63	1.43
1	CC3	14.00	6.45	1.21
2	CC1	20.20	6.13	2.06
2	CC2	19.00	7.63	1.74
2	CC3	18.50	9.75	1.14
3	CC1	23.30	6.58	2.33
3	CC2	22.00	11.15	1.44
3	CC3	26.00	11.68	1.04
4	CC1	22.60	10.70	1.81
4	CC2	21.20	12.33	1.40
4	CC3	18.90	10.78	1.20
5	CC1	20.60	12.23	1.38
5	CC2	21.00	10.40	1.39
5	CC3	19.30	18.55	0.53

		WALL THICKNESS (u)	DURATION OF THICKENING (days)	RATE OF THICKENING (u/day)
1	CC1	2.46	11.03	0.24
1	CC2	2.70	20.70	0.13
1	CC3	2.60	30.03	0.09
2	CC1	2.80	15.43	0.20
2	CC2	2.40	30.60	0.09
2	CC3	2.60	36.65	0.08
3	CC1	3.20	24.13	0.14
3	CC2	2.90	34.90	0.09
3	CC3	2.90	40.60	0.05
4	CC1	3.30	32.00	0.12
4	CC2	3.10	39.50	0.08
4	CC3	3.10	65.00	0.05
5	CC1	3.70	40.00	0.10
5	CC2	3.30	38.90	0.08
5	CC3	3.10	63.90	0.05

CELL WALL THICKNESS OF LAST FORMED TRACHEIDS

For all stem positions, tracheid wall thickness increased across the growth ring (Figure 9). In CC1, this increase was more rapid with successive positions down the stem, so that at comparable times within the season, cell wall thickness increased down the stem.

In CC2 and CC3, apart from a tendency toward thinner cell walls later in the season in the two upper stem positions, there was no obvious trend among positions. Cell wall thickness was greater in CC1 at the beginning of the growth ring than in either CC2 or CC3. However, the thickness attained was comparable in all crown classes at the end of the season. Differences among stem positions and crown classes were not significant (Table 5).

DURATION OF SECONDARY WALL DEPOSITION

For all positions, duration of wall deposition increased through the season (Figure 10). The seasonal increase in duration was more rapid at successively lower stem positions resulting in a clear trend of increasing duration down the tree. Stem positions had a

highly significant effect on the duration of secondary wall thickening ($p<0.0001$).

Among crown classes, mean durations of cell wall thickening were greatest in CC3 and least in CC1 (Table 6). These differences were shown to be highly significant ($p<0.0001$).

RATE OF SECONDARY WALL DEPOSITION

Rate of secondary wall deposition varied very little throughout the season in CC2 and CC3 (Figure 11). In CC1, the rate of wall deposition tended to decrease to a minimum at about mid to late May, after which time, it increased in the upper two stem positions and remained constant at other positions.

In all crown classes, rate of wall deposition was maximum in the uppermost stem position (Figure 11). In CC1 there was a trend of decreasing rate down the stem. In CC2 there were no differences among the four lower stem positions, and in CC3, no differences among the three lowermost stem positions.

Analysis of variance showed highly significant ($p<0.0001$) differences in the rate of wall deposition among crown classes (Table 5). However, differences occurred only between the uppermost stem position of CC1

and CC3, and CC2 and CC3. Otherwise, CC2 and CC3 were similar. The three lowermost stem positions of CC1 were similar from those of CC2 and CC3.

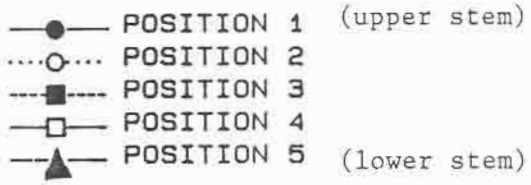
FIGURES 5 TROUGH 11

SEASONAL KINETICS OF TRACHEID DIFFERENTIATION IN DOUGLAS FIR

DAY OF THE FIRST SAMPLE = DAY 0, 28 FEB 86
DAY OF THE LAST SAMPLE = DAY 160, 7 AUG 86

CC1 - open-grown trees
CC2 - stand-grown codominant trees
CC3 - stand-grown suppressed trees

KEY TO STEM POSITIONS:



Placement of symbols on figures represents time at which fifth successive tracheids were produces through the season.

FIG. 5: DURATION IN THE CAMBIAL ZONE

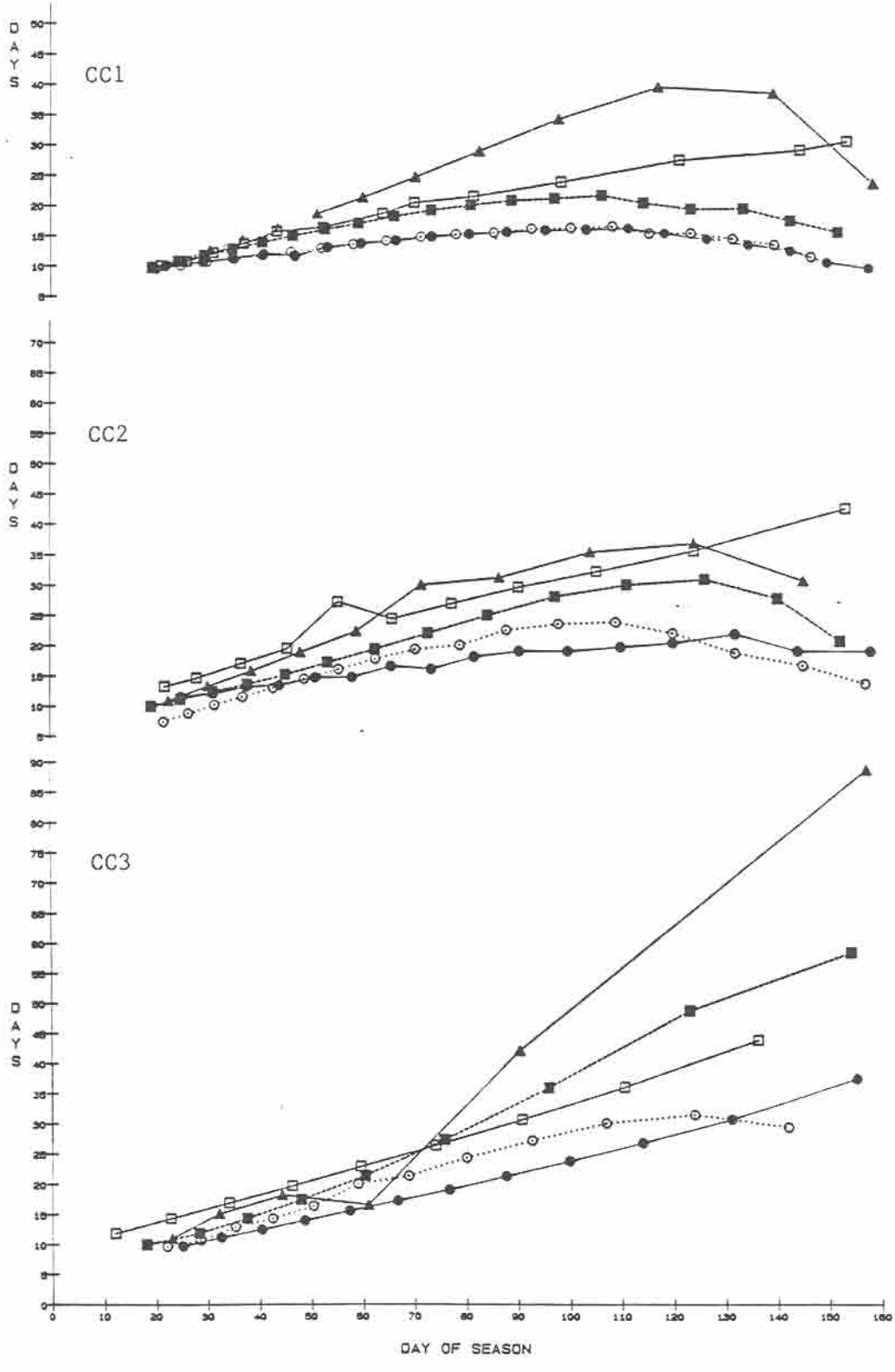


FIG. 6: MAXIMUM RADIAL CELL DIAMETER

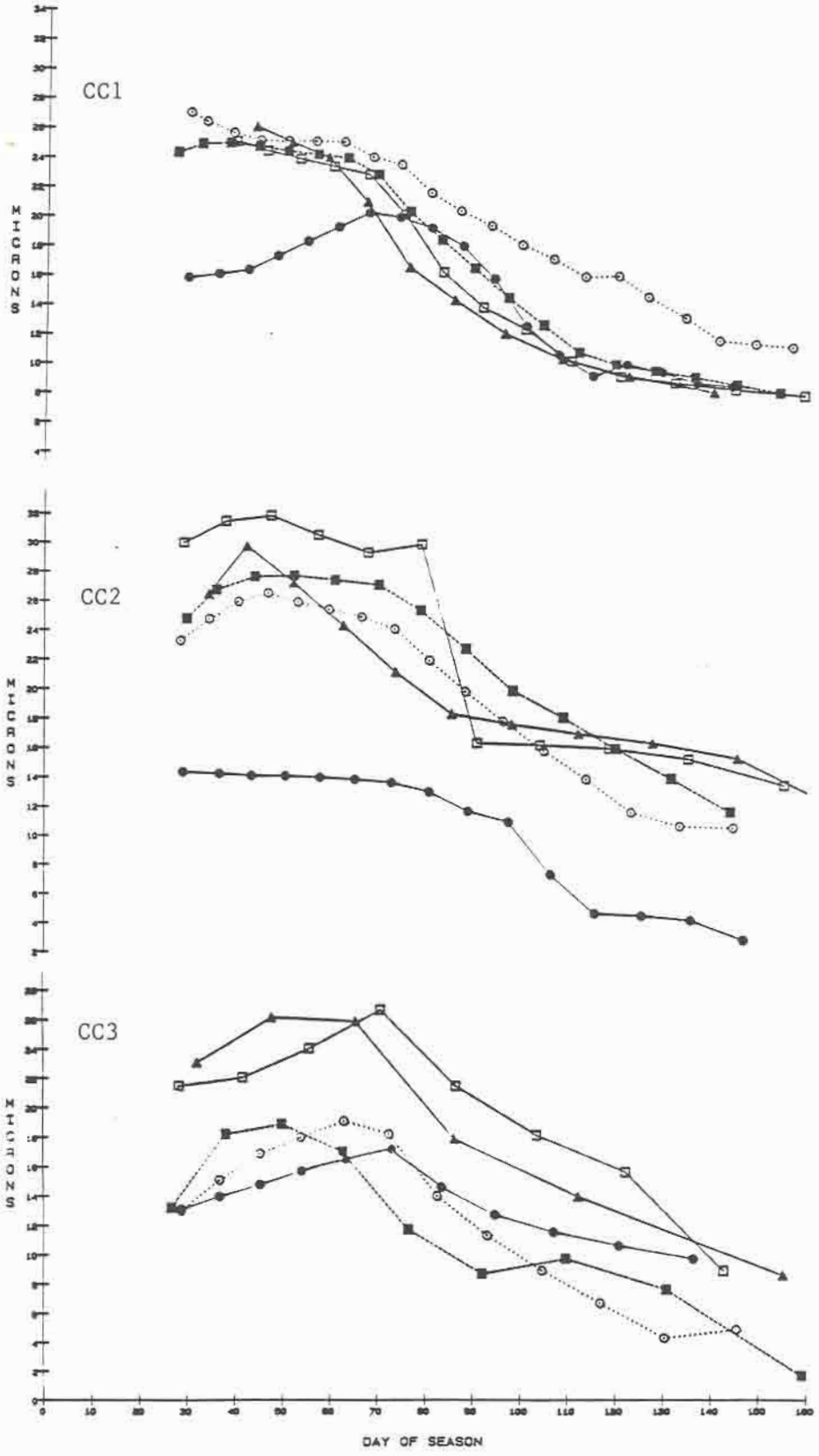


FIG. 7: DURATION IN THE EXPANDING ZONE

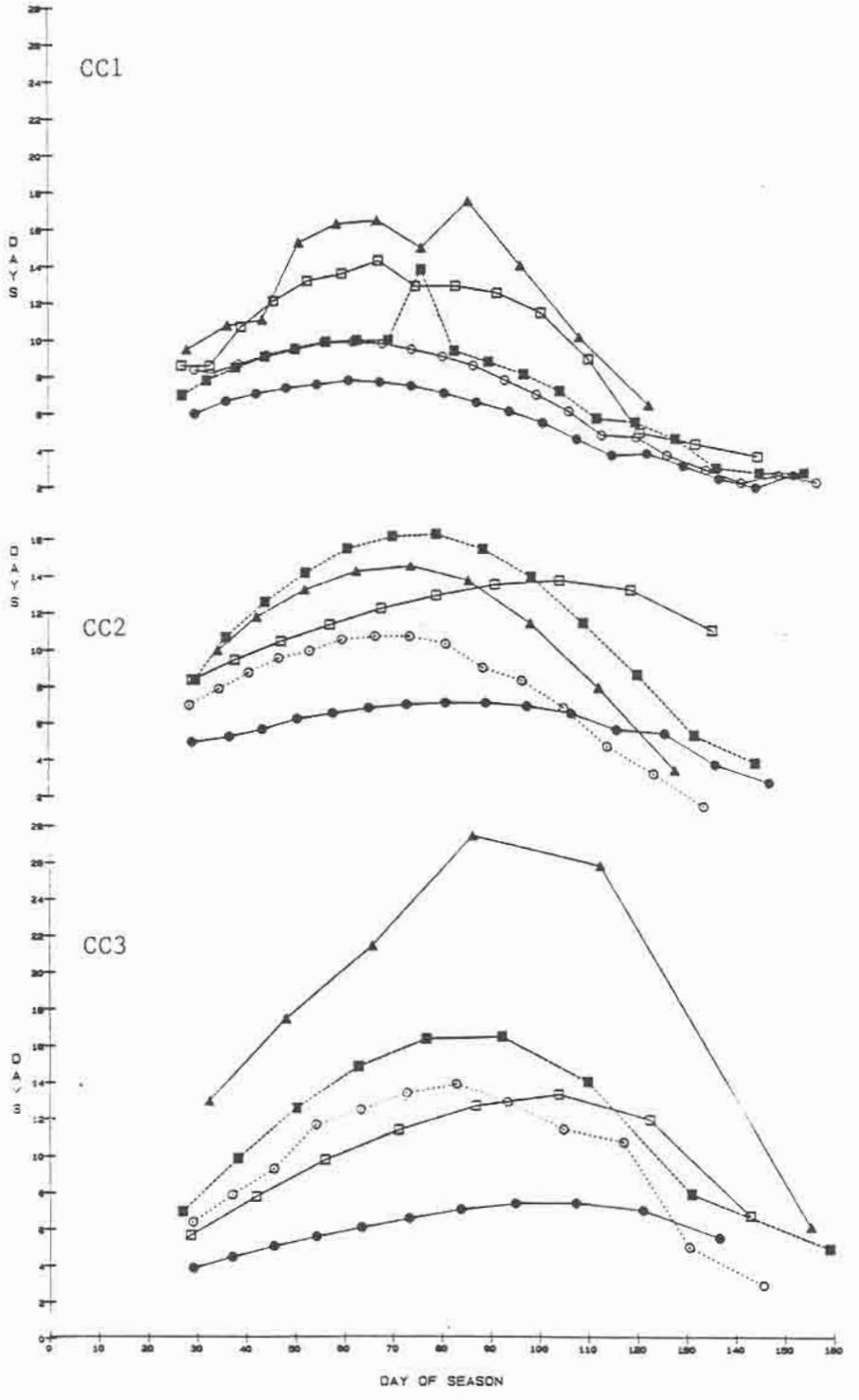


FIG. 8: RATE OF RADIAL CELL EXPANSION

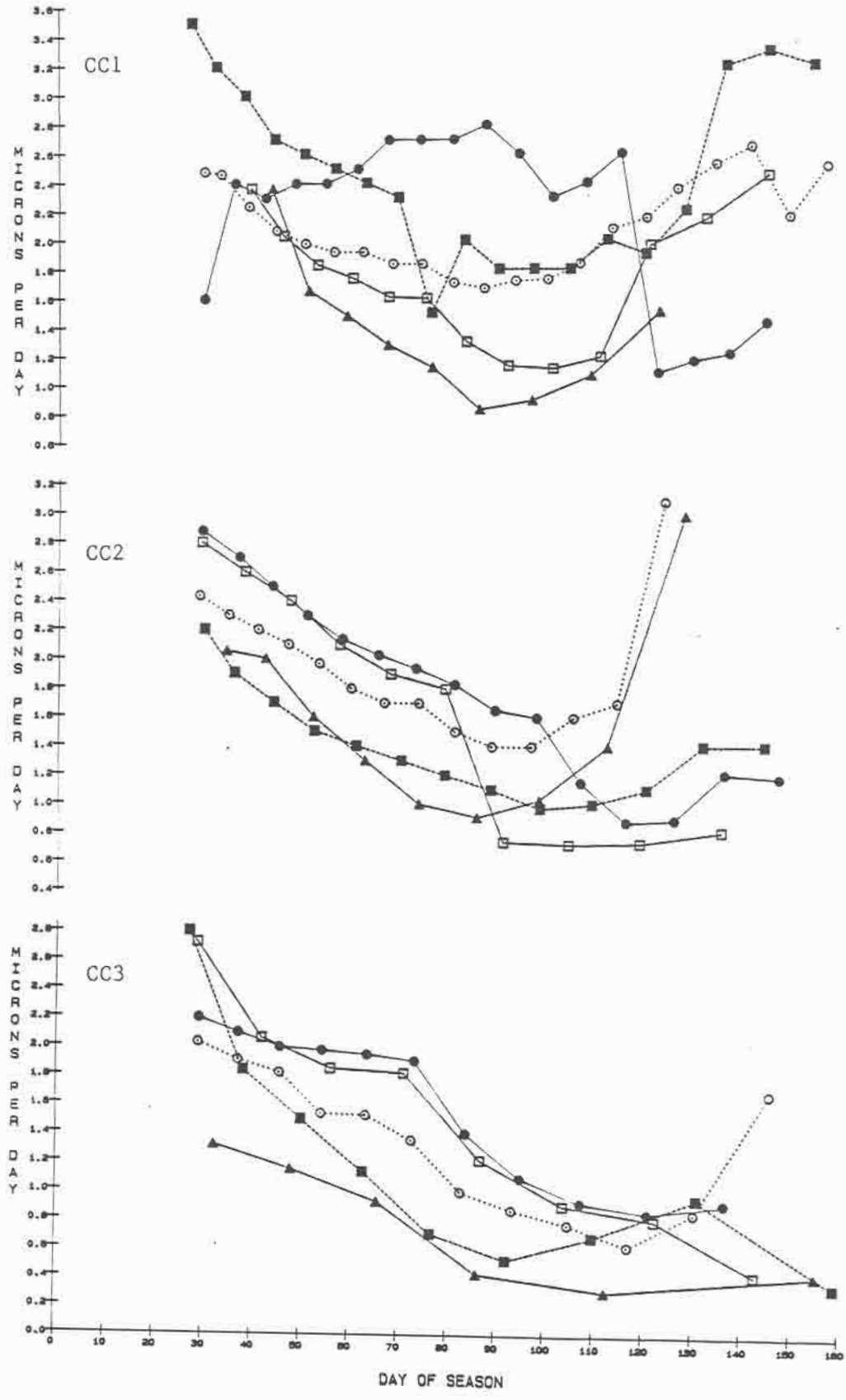


FIG. 9: CELL WALL THICKNESS

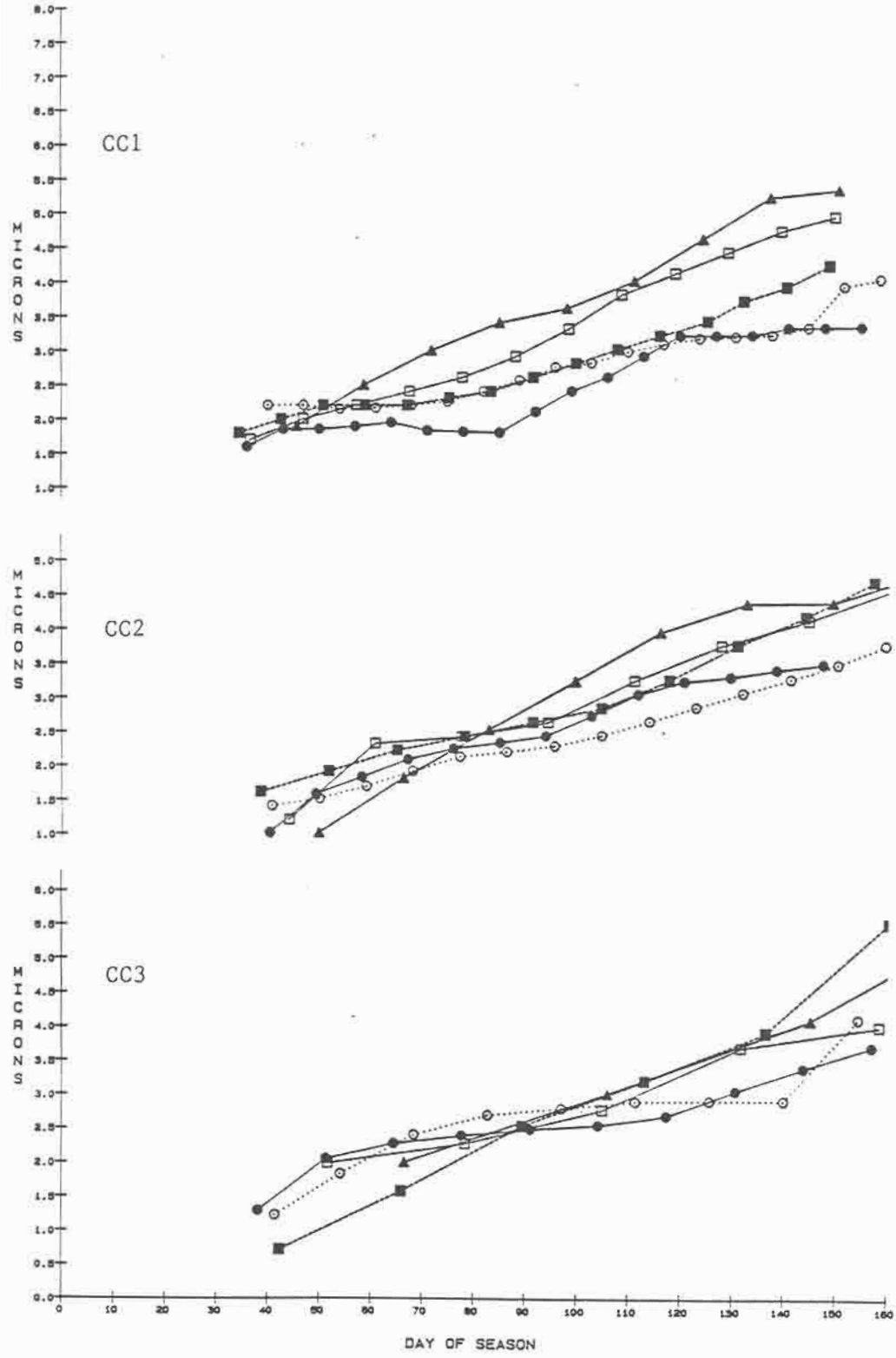


FIG. 10: DURATION OF CELLS IN WALL DEPOSITION

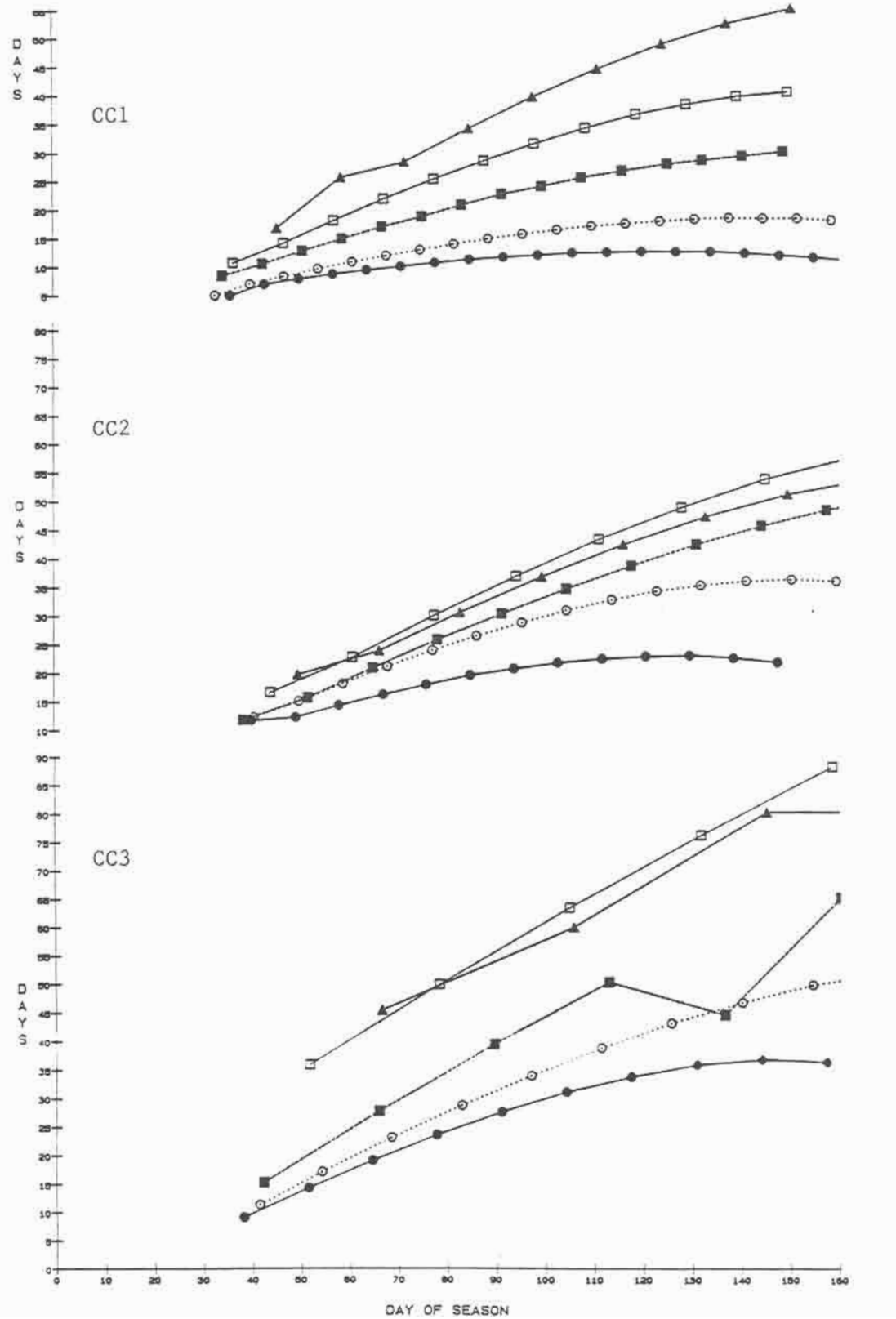
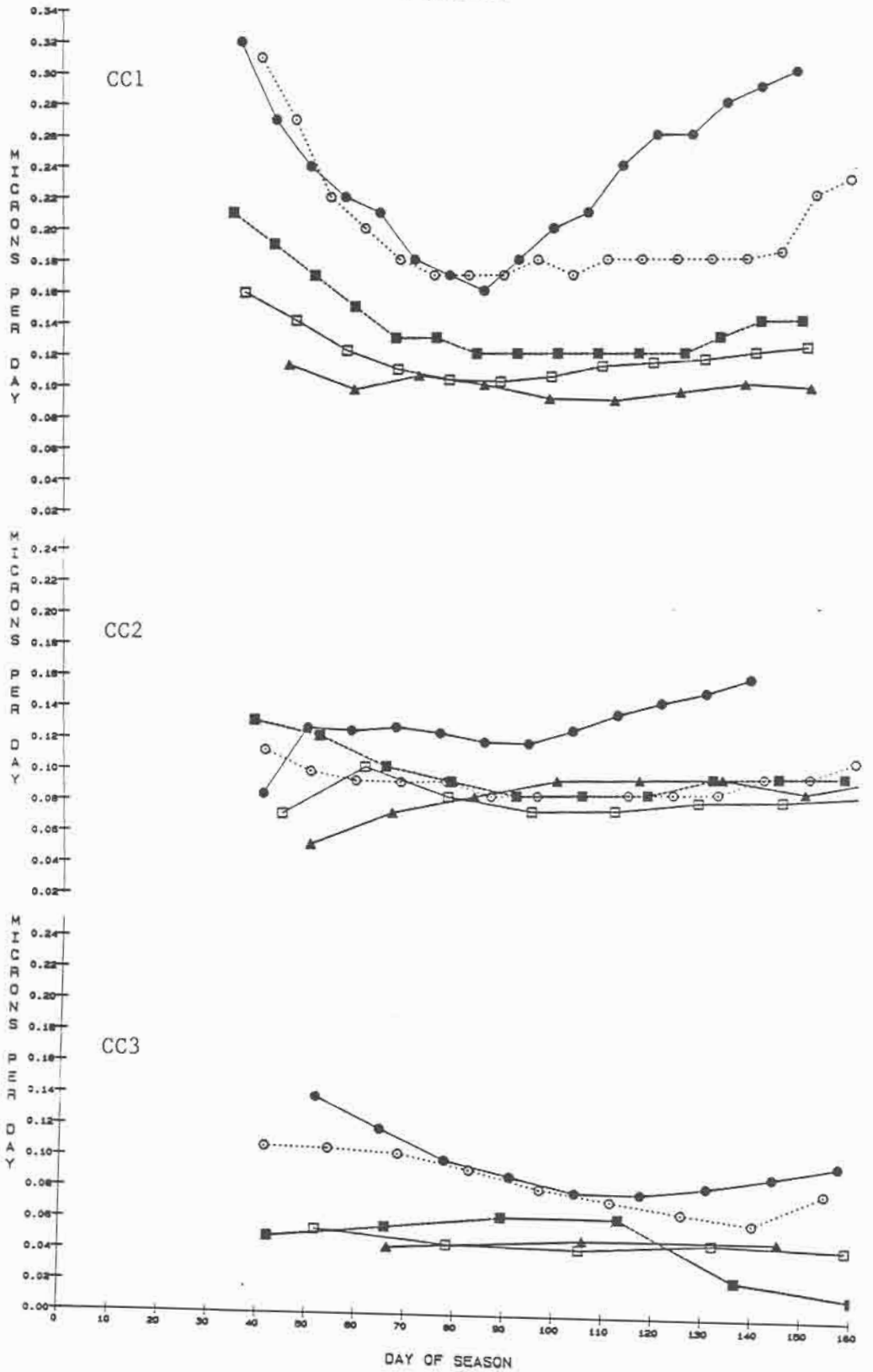


FIG. 11: RATE OF CELL WALL DEPOSITION



DISCUSSION

CAMBIAL REACTIVATION

Cambial reactivation was judged by the appearance of radially expanding cells. On the first harvest date, radially expanding cells were found in the uppermost positions of all trees, and basally to positions roughly corresponding to the site of the lowermost branches in crown classes 2 and 3 (Table 3). Therefore, within each stem, the observed pattern is consistent with the concept of basipetal spread of cambial reactivation.

Cell wall deposition also began earliest in the uppermost stem positions (Table 3) and also seemed to spread basipetally. By the time deposition was well underway throughout the stem, larger numbers of cells were found in this phase in lower stem positions. Since fewer cells were produced at more basal stem positions, a higher percentage of the cells produced were in this phase early in the season than higher in the stem.

Wall deposition had begun by day 14 in CC1 and CC2, but did not commence in CC3 until around day 28. Therefore, differentiation was proceeding more slowly in more suppressed trees.

SEASONAL TRACHEID DIFFERENTIATION

The seasonal change in radial cell diameter was more closely correlated with the duration of radial expansion than with its rate (Figures 6 and 7). A steady decline in cell diameters from early to mid May was correlated with a decline in the duration of expansion. During this time, rate of cell expansion showed relatively little change.

Prior to May, cell diameters increased slightly or remained relatively constant. At this time of the season, durations were increasing and rates decreasing. This may suggest that the control of radial expansion is not constant throughout the season, with a greater influence of rate in the early season. Wodzicki (1971) suggested that the rate of cell expansion is correlated with temperature. Perhaps cells differentiating early in the season can respond more readily to environmental conditions. Zajaczkowski (1973) has shown seasonal differential response to several growth factors.

The results here contradict Larson's hypothesis that decreasing IAA concentrations after cessation of shoot growth lead to decreasing cell diameters. The decline in duration of cell expansion at a time that shoot growth had ceased is not consistent with decreasing auxin concentrations since low IAA levels are

commonly associated with a delay in differentiation (Porandowski, et.al., 1982; Wodzicki and Wodzicki,1980; Aloni and Zimmerman,1983). Wodzicki (1971) reported no change in IAA concentration throughout the season and it has been suggested that the changing response of cells to IAA is more important than IAA concentrations present in the tissue (Zajaczkowski, 1973).

Axial gradients in cell dimensions have been reported by many authors. Results here confirm a general trend for increasing cell diameter and cell wall thickness down the stem, although this was not always consistent among crown classes or through the season. The greatest differences in radial diameter of cells among stem positions were in the more suppressed trees, whereas greatest differences in cell wall thickness were in the most vigorous trees. This suggests that the mechanism of regulation of axial patterns of cell diameter and wall thickness is different. Dodd (1985) suggested that some anatomical variables are more closely related to the tree's crown than others. This could be a factor in explaning the differences in regulation of these two processes.

In CC2 and CC3 trees, the basal increase in cell size appeared to be due to longer durations of cell expansion since rates of expansion tended to be greatest near the top of the tree. However, in the more vigorous

trees of CC1, the influence of rates of expansion were more important than in the other two crown classes, resulting in little trend in cell size down the tree. The evidence here tends to suggest that a pattern of duration along the stem is more fixed than is the rate of cell expansion. The latter may be influenced by the supply of substrates from the crown. This would explain the overall higher rates of cell expansion in the more vigorous trees. It may also explain higher rates of expansion in the upper stem of suppressed trees with higher crowns which was not true of the full-crowned trees. The supply of carbohydrates in a stem have been shown to affect rate processes (Denne, 1979).

The regular axial increase in duration of expansion down the tree is consistent with the hypothesis of Aloni and Zimmermann (1983). Presumably, auxin concentrations decline down the stem. If this is true, then duration of cell expansion would be maximum at lower stem positions as shown here. It might also be expected that differences in auxin concentration down the stem would be greatest in suppressed trees. This would be consistent with the greater differences in duration of cell expansion along the stem observed in suppressed trees here.

The seasonal increase in cell wall thickness was closely correlated with the duration of wall thickening.

There was relatively little variation in rate, and where rate of wall thickening did vary, this was, again, most pronounced in the early part of the season. These results are consistent with previous work showing seasonal change in wall thickness to be determined by the duration, rather than the rate, of wall thickening (Wodzicki, 1971; Skene, 1972: Denne, 1979). Again, the pattern in duration along the stem is fixed. Differences in duration along the stem are greater in CC3. If auxin declines down the stem as proposed by Aloni and Zimmermann (1983), and if the lower basipetal concentration of auxin produces a increase in the duration of wall deposition as shown by Nix and Brown (1987), an auxin gradient could explain wall deposition processes as it could for processes of radial expansion.

It is possible that higher rates of wall deposition are associated with greater availability of substrate. Rates of wall deposition were higher in CC1 and in the uppermost stem position of CC2 and CC3, coincident with foliage. However, final wall thickness attained did not vary among crown classes and did not vary significantly between most stem positions. Longer durations observed here did not seem to produce thicker tracheid walls as longer duration of cell expansion had produced larger diameter cells. Perhaps final wall thickness is dependent on the rate of wall thickening since this rate

also varied little between all lower stem positions of the three crown classes. However, measurements of wall thickness were linear and did not reflect the volume of wall material per tracheid. Since radial diameters decrease somewhat down the stem and toward more suppressed trees, an equal volume of wall material would have made thinner walls on a larger diameter cell. The seeming lack of correlation of final wall thickness with duration of secondary wall deposition could have been confounded by the lack of consideration of cell diameter differences among crown classes. Cells in CC3 which were smaller attianed the same linear wall thickness as those in CC1, but since their diameters were smaller, they produced more wall volume. Thus, it is more likely that final cell wall thickness correlates with the duration rather than the rate of wall thickening.

CONCLUSIONS

The duration that cells spend in the cambial zone and in both differentiating phases showed a clear trend to increase basipetally in all crown classes. This trend seemed to be fixed, thus durations are likely more influenced by some age-related factor than by crown class. Crown class seems to have a quantitative effect

on durations rather than an influence on relative durations from one stem height to another. While rates varied little between stem positions in any one crown class, crown classes were highly significantly different, therefore, the same quantitative effect of crown class on rate exists as for duration. If it can be assumed that durations at various stem levels are dependent on an age-related factor, rates are not. Rather, within a stem and through a growing season, rates may be more dependent on environmental factors, such as temperature.

The findings of this work may be useful in forest management practices. A treatment increasing the depth of the crown, such as fertilization or growth in open stands, would be expected to produce more uniform cell sizes and increase wood production throughout the stem. However, this would also be expected to result in a greater proportion of earlywood throughout the stem and to produce wood with juvenile characteristics through a number of growth rings from the pith. It appears no type of intervention could be expected to produce greater consistency through the stem in diameters and wall thicknesses of cells due to their correlation with durations which are fixed by either age of the apical meristem at the time the cambium that produces them is formed, or age of the cambium at the time its cells

differentiate into wood elements. Thus, pruning a vigorous tree's lower branches without significantly reducing the assimilate supply may be the best way to obtain a greater amount of latewood throughout the bole, to maintain consistency in cell size, and to reduce the number of years juvenile wood is produced without sacrificing the quantity of wood produced.

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